

# Sticky Surfaces: Adhesion Based Microbial Aggregation Dynamics

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We consider spherical particles coated with binding ligands and study their attachment in quiescent flow. This fluid-immersed adhesion is widespread in many biological and artificial settings. Our theory highlights how the physics of the binding kinetics of these ligands (expressed through the collision factor function) as well as the attractive / repulsive surface potential in an ionic medium effects the eventual size of these particle aggregates (flocs). For experimentally measurable parameters in microbial population studies, our results suggests that large floc aggregates are possible with more stretchable ligands due to efficient inter-floc collisions (or a large, non-zero collision factor). Strong electrolytic composition of the surrounding fluid favors large floc formation as well.

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*Introduction* – The formation of aggregates, induced by the adhesion of two spherical particles or nearby surfaces is important in many scientific and industrial processes. In particular, interfacial attachment leading to larger floc aggregates via the latching of binders on surfaces in close proximity is widespread. Examples include binding of bacterial clusters to medical implants and host cell surfaces [1], cancer cell metastasis [2], and the coalescence of medical gels with nano-particles for targeted drug delivery [3]. Moreover, coagulation and flocculation (the chemical and the physical aspects of adhesion) are also important in pulp and paper-making industries as well as waste water treatment plants [4]. Although the microscopic structure and geometry of the adhering surfaces of flocs in these phenomena vary greatly, they share a common underlying physics. Past investigations in theoretical modeling of fluid-borne surface adhesion have addressed ligand-receptor binding kinetics [5, 6], surface deformation [7, 8] and flow past the surrounding surfaces [9, 10]. Our aim in this letter is to explore how this adhesion (*collision* in colloid science literature) mechanism for spherical flocs is governed by various geometric and fluid parameters as well as how the surface forces and binding kinetics of the ligands impact the eventual sizes of these flocs.

*Model* – Our future studies are geared towards tracking bacterial aggregates in suspension, as a continuum mass of EPS (extracellular polymeric substance) network [11]. Following the general outline given in [12], we define

$b(t, x, \mathbf{s})\Delta x$  = number of bacterial biomasses having volumes between  $x$  &  $x + \Delta x$  in time  $t$  at a spatial point  $\mathbf{s}$ .

In volumes between  $x_1$  and  $x_2$ , the total number of flocs  $B_0$  is given by

$$B_0(t, x_1, x_2, \mathbf{s}) = \int_{x_1}^{x_2} b(t, x, \mathbf{s}) dx \quad (1)$$

for  $[x_1, x_2] \subset [\underline{x}, \bar{x}]$ , where  $\underline{x}$  and  $\bar{x}$  are the minimum and maximum aggregate volume sizes, respectively. A finite nutrient supply and the duration of the experiment allow us to assume that  $\bar{x}$  is finite. Further, the extracellular structures must be actively maintained and thus the minimal size  $\underline{x}$  is the volume of one bacteria. The conservation of bacterial biomass through aggregation, or the governing equation for  $b$  is [12]

$$b_t + \mathbf{v} \cdot \nabla_{\mathbf{s}} b = A(x, b) \quad (2)$$

where  $\mathbf{v}$  is the relative velocity of one floc with respect to the other.  $A = A_{\text{in}} - A_{\text{out}}$ , where  $A_{\text{in}}$  is the rate with which flocs of size in  $[x, x + \Delta x]$  are created and  $A_{\text{out}}$  is the rate a floc of size in  $[x, x + \Delta x]$  joins with another floc, to form a volume greater than  $x + \Delta x$ . These rates are given by

$$A_{\text{in}}(x, b) = \frac{1}{2} \int_{\underline{x}}^{x-\underline{x}} K_A(y, x-y) b(t, y, \mathbf{s}) b(t, x-y, \mathbf{s}) dy, \quad x \in [2\underline{x}, \bar{x}] \quad (3a)$$

$$A_{\text{out}}(x, b) = b(t, y, \mathbf{s}) \int_{\underline{x}}^{x-\underline{x}} K_A(x, y) b(t, y, \mathbf{s}) dy, \quad x \in [\underline{x}, x - \underline{x}] \quad (3b)$$

$K_A$  is the aggregation kernel, describing the rate with which flocs of volume  $x$  and  $y$  combine to form a floc of volume  $x+y$ . The next two sections will focus on modeling this kernel based on the hydrodynamics and surface potentials of two coalescing spherical floc-surfaces.

*Adhesion mechanics* – We present a simple model of interfacial attachment between two spheres (of radius  $R_1$  and  $R_2$ , Fig.1) immersed in a fluid medium [5]. The surface of the spheres bind onto each other due to the presence of adherent elastic binders (polymer strands with sticky heads) on the surfaces, as well as the attractive surface potential. The adherent binders are idealized as linear Hookean springs with stiffness  $\kappa_0$  and mean rest length  $l_0$  (Fig.1). The effect of the shear flow on the mean rest

length of the binders, as well as the shearing effect of the mean flow on the binders is neglected [13]. These simplifications allow us to focus on the dynamics normal to the surface, but could be relaxed for a more general case[7].

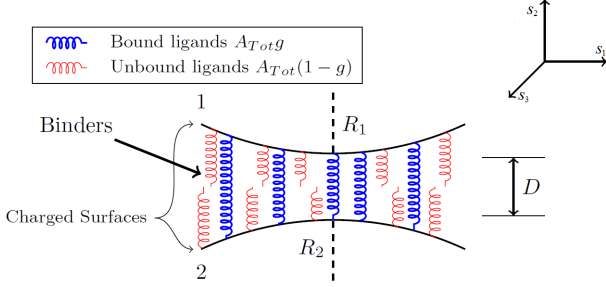


FIG. 1: Two polymer coated coalescing spherical flocs.

For a given spatial point  $\mathbf{s} = (s_1, s_2, s_3)$ , define  $A_{\text{Tot}}g(\mathbf{s}, t)dA$  as the number of bonds in the transverse direction that are attached between the surfaces  $dA$  at time  $t$ ,  $D(\mathbf{s})$  be the minimum distance between the two spheres,  $A_{\text{Tot}}$  be the total number of binding ligands and  $g$  be the density of bound ligands on the adhesion surface. In floc literature, the function  $g$  is synonymous to the collision factor as well. Hence the total number of bonds formed in the transverse direction is  $\int_{A_c} A_{\text{Tot}}g(\mathbf{s}, t)dA$ , with  $A_c$  being the area of adhesion. The bond attachment/detachment rates, influenced by the surface potential of the two charged surfaces, are

$$K_{\text{on}}(\mathbf{s}) = K_{\text{on}}^* \exp \left[ \frac{-\kappa_s(D(\mathbf{s}) - l_0)^2 + W(D(\mathbf{s}))}{2k_B T} \right]$$

$$K_{\text{off}}(\mathbf{s}) = K_{\text{off}}^* \exp \left[ \frac{(\kappa_0 - \kappa_s)(D(\mathbf{s}) - l_0)^2 + W(D(\mathbf{s}))}{2k_B T} \right] \quad (4)$$

where  $k_B$  is the Boltzmann constant,  $T$  is the temperature,  $\kappa_s$  is the spring constant of the transition state used to distinguish catch ( $\kappa < \kappa_s$ ) from slip ( $\kappa > \kappa_s$ ) bonds [5],  $W(D)$  is the total surface potential,  $K_{\text{on}, \text{eq}}^*$ ,  $K_{\text{off}, \text{eq}}^*$  are the equilibrium binding affinities. For notational simplicity, we denote  $D(\mathbf{s}) \equiv D$ . In the limit of small binding affinity and abundant binding receptors on the surface of adhesion ( $A_{\text{Tot}}K_{\text{on}, \text{eq}}/K_{\text{off}, \text{eq}} \ll 1$ ), the bond ligand density evolves as the following differential equation [5]:

$$\frac{dg}{dt} = A_{\text{Tot}}K_{\text{on}} - K_{\text{off}}g, \quad (5)$$

where  $\frac{dg}{dt} = \frac{\partial g}{\partial t} + \mathbf{v} \cdot \nabla_{\mathbf{s}} g$ . The instantaneous force that these two colliding charged surfaces exert on each other (and acting normal to the surface) becomes:

$$\mathbf{f}(\mathbf{s}, t) = \kappa_0(D - l_0) + \nabla_{\mathbf{D}} \cdot W(D) \quad (6)$$

where the first term represents the stretching force due to Hooke's law and the second term represents the forces

due to the surface potential. The direction of this force is along the direction vector from the spherical floc of radius  $R_1$  to the floc of radius  $R_2$ . The total force arising from all such bonds is given by

$$\mathbf{F}(\mathbf{s}, t) = A_{\text{Tot}} \int_{A_c} g(\mathbf{s}, t) \mathbf{f}(\mathbf{s}, t) dA(\mathbf{s}, t) \quad (7)$$

In the limit of small binding affinity and normal adhesion, the adhesion area,  $A_c$ , is given by  $A_c = \pi R_c^2$  [13], where the adhesion radius,  $R_c$ , is

$$R_c(\mathbf{s}, t) = \left( \frac{2k_B T}{\kappa_0} \right)^{1/4} l_0 (R_1^{-1/2}(\mathbf{s}, t) + R_2^{-1/2}(\mathbf{s}, t)) \quad (8)$$

Finally, in a Stokes flow, the aggregation rate,  $K_A$ , is

$$K_A = \gamma_A A_c \mathbf{F} / \zeta \quad (9)$$

$\zeta$  is the drag coefficient,  $\gamma_A$  is the aggregation contact efficiency parameter. Eqns. (3a, 3b, 2, 5, 6, 7, 9) along with initial conditions,  $b(0, x) = b_0(x)$ , is the entire system.

*Long range interactions* – Experiments are being performed to determine the physiochemical characteristics of two coalescing, polymer-coated, charged bacterial flocs immersed in ionic solvents [14]. We describe these interactions through the DLVO approach, i.e., the Coulombic and Van der Waals interaction. To simplify modeling framework, other interactions including hydration effects, hydrophobic attraction, short range steric repulsion and polymer bridging are neglected [15].

For two charged spheres, with radii  $R_1, R_2$ , the repulsive Coulombic forces in the gap,  $D$ , is given by

$$W_C(D) = 2\pi\epsilon_0\epsilon\psi_0^2 k_B T \left( \frac{2R_1 R_2}{R_1 + R_2} \right) e^{-\kappa D} \quad (10)$$

where  $\kappa$  is the Debye length,  $\epsilon, \epsilon_0$  are the dielectric constant of vacuum and the medium, respectively,  $\psi_0$  is the *zeta potential* or the electric potential of the diffuse cloud of charged counterions. The attractive Van der Waals forces for spherical flocs in the regime of close contact ( $D \ll R_1, R_2$ ), is

$$W_{\text{VW}}(D) = -\frac{Ak_B T}{6D} \frac{R_1 R_2}{R_1 + R_2} \quad (11)$$

where  $A$  is the Hamaker constant, measuring the van der Waal 'two-body' pair-interaction for macroscopic objects. The net surface potential is  $W(D) = W_C(D) + W_{\text{VW}}(D)$ . This potential is pair-wise attractive over very short and long distances, and pair-wise repulsive over intermediate distances (Fig.2).

*Results* – We consider the case of homogeneous floc aggregation (the variables are independent of the spatial location,  $\mathbf{s}$ ), under quiescent flow conditions. If the adhesion-detachment rate of the flocs is sufficiently rapid so that they remain in equilibrium, the fluid surrounding the

flocs is stagnant and the effects of peeling velocity is negligible [5], then the bond-ligand density (i.e. the collision factor),  $g$ , (Eqn. 4, 5) evolves according to

$$g = A_{\text{Tot}} \frac{K_{\text{on}}}{K_{\text{off}}} = \frac{A_{\text{Tot}} K_{\text{on}}^*}{K_{\text{off}}^*} e^{-\kappa_0 \frac{(D-l_0)^2}{2k_B T}}, \quad (12)$$

and the total adhesion force between two flocs reduces to

$$\mathbf{F}(t) = A_{\text{Tot}} g \mathbf{f}[\pi R_c^2(t)] \quad (13)$$

The parameters used in simulations are listed in Table I.

Parameter	Value	Units	Source
$\kappa_0$	$(0.01-10) \times 10^{-3}$	$\text{N m}^{-1}$	[13]
$l_0$	$10^{-8}$	m	[13]
$\zeta$	(0.01-2.5)	$\text{N m}^{-1} \text{s}$	—
$A_{\text{Tot}}$	$10^9$	$\text{m}^{-2}$	[13]
$K_{\text{on}}^*/K_{\text{off}}^*$	$10^{-12}$	—	[13]
$\gamma_A$	$2.7 \times 10^{-15}$	$\text{fL}^{-2}$	[12]
$\epsilon_0$	$8.854 \times 10^{-12}$	$\text{Fm}^{-1}$	—
$\epsilon$	78.5	—	—
$\psi_0$	25	mV	[15]
$\kappa$	1.5	$\text{nm}^{-1}$	[15]
$A$	2.0	—	[15]

TABLE I: Parameters common to all simulations.

*Surface potential and collision factor* – The pair-wise surface potential,  $W(D)$ , is valid over short distances ( $D \ll R$ ). The electrolyte is assumed to be a 1-1 electrolyte at a concentration of 50 mM, zeta potential of 25mV and Debye length for screened charges  $\kappa^{-1} = 1.5 \text{ nm}^{-1}$ , a typical of values for particles in water [16].

With increasing binder stiffness, the potential energy barrier as well as the depth of the secondary minima increases (Fig. 2a). Hence aggregation becomes less favorable with increasing binder stiffness, a fact seen in Fig. 4a, as well. However, at critical separation distances ( $5 \leq D \leq 12 \text{ nm}$ ), the depth of the secondary minima remains lower than the average thermal energy of the particles ( $3k_B T/2$ ), implying that at this range the aggregating flocs are colloidally stable. The primary minima shown here is unphysical, since at very short separation distances the non-DLVO repulsive steric interaction is dominant that prevents the particles from coming into true contact. The regions of attraction/repulsion of this potential is inferred from surface force,  $\mathbf{f}$  (Fig. 2b). At intermediate distances ( $1 \text{ nm} < D < l_0$ ), the repulsive Coulombic forces are dominant while at long distances ( $D > l_0$ ), the adhesive forces are dictated by the attractive spring force of the stretched binders. We choose  $D^* = 11 \text{ nm}$ , where the adhesive forces are attractive.

The collision factor,  $g(D)$ , (where the separation distance,  $D = \frac{s_1^2 + s_2^2}{R} + l_0$ , with the center of the spatial frame, fixed at the point of minimum separation on sphere 2,  $R$  being the radius of identical colliding flocs, see Fig. 1) is symmetric about the mean rest length of the binders,  $l_0$ ,

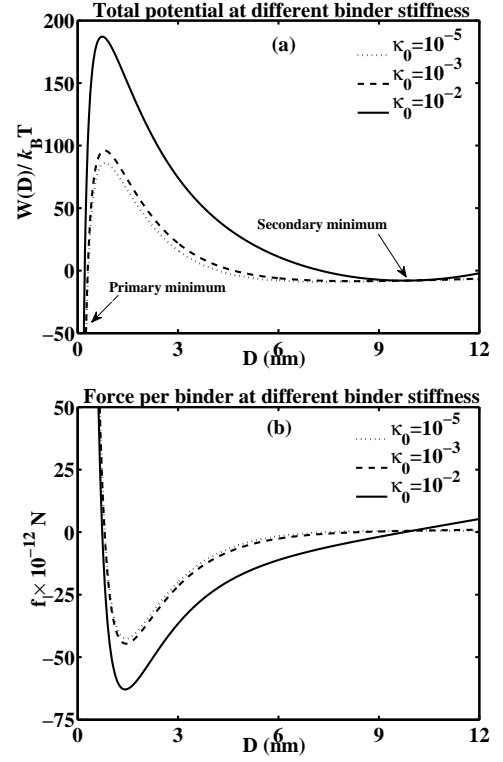


FIG. 2: (a) Total pairwise potential,  $W(D)$ , versus the floc separation distance,  $D$ , for two identical spherical microbial flocs with varying binder stiffness but fixed size,  $R=1\mu\text{m}$ , (b) Surface potential force,  $\mathbf{f}$ , for different binder stiffness, showing regions of attraction ( $\mathbf{f} > 0$ ) and repulsion ( $\mathbf{f} < 0$ )

in the absence of fluid flow (Fig. 3). Since the floc separation distance with a significant non-zero contact is considerably reduced for stiff binders, the adhesion-detachment mechanism is more efficient for tensile springs.

*Numerics* – To solve our model, we employed the discretization scheme developed by Banks [17] and adopted by Doumic [18]. The convergence of the scheme was tested using the test functions in [12]. A linear relationship between the  $L^\infty$ -error and the mesh-size,  $\delta x$  was found using this first order approximation scheme. The initial number density is chosen as  $b_0(x) = 7.47 \times 10^{-4} e^{-0.00676x}$ , where the coefficients are fit to the experimental data from the Younger Lab [12]. All solutions are marched in time until  $T=100$  minutes. We chose 1 femtoliters (fL) as a lower bound  $\underline{x}$  in our simulations. Our aggregation model allows the upper bound,  $\overline{x}$ , of the domain to go unrestrained (i.e.,  $\overline{x} \rightarrow \infty$ ), but the results are presented inside the window  $1 \leq x \leq 1000 \text{ fL}$ .

In this letter we limit our parameter analysis to binder stiffness and the electrolytic composition of the fluid medium. Fig. 4a suggests that stiff binders lead to fewer large aggregates (i.e.,  $b(x, T, \kappa_0 = 10^{-2}) < b(x, T, \kappa_0 = 10^{-3}) < b(x, T, \kappa_0 = 10^{-5})$ , for  $x \geq 600$ ). Since aggregation is influenced by the collision factor, a higher value of  $g(D^*)$  suggests that two flocs close to each other

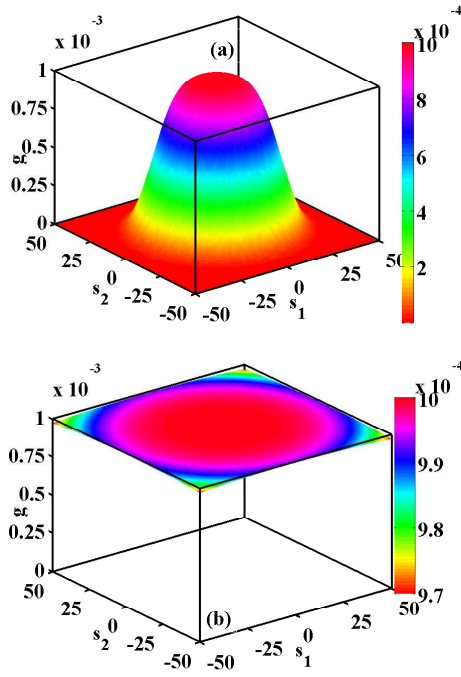


FIG. 3: Collision factor  $g$  vs spatial coordinates  $(s_1, s_2)$  for identical colliding flocs of radius  $R=1\mu\text{m}$  with (a)  $\kappa_0 = 10^{-2} \text{ Nm}^{-1}$  and (b)  $\kappa_0 = 10^{-5} \text{ Nm}^{-1}$ . Stiff ligands have a narrow range of separation distance  $D(s_1, s_2)$  with a non-zero collision impact.

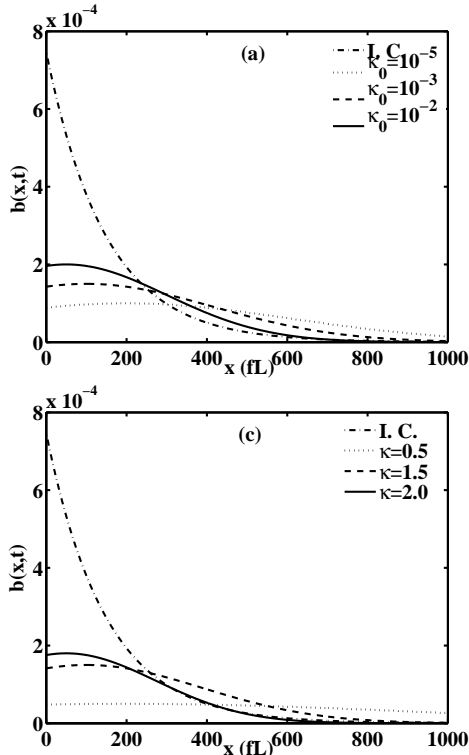


FIG. 4: Floc number density distribution versus floc-volume at (a) different binder stiffness and screening length,  $\kappa = 1.5$ , and (c) different screening lengths and  $\kappa_0 = 10^{-3} \text{ Nm}^{-1}$ . The dash-dot curve in these figures is the initial conditions ( $b_0(x)$ ). are more likely to coalesce (Fig. 3). This factor is greatly

compromised at a floc separation distance,  $D^* = 11\text{nm}$ , for stiff binders, leading to fewer large aggregates. Similarly, the number of large aggregates drops for a weaker electrolytic fluid composition (or longer Debye length,  $\kappa$ , Fig. 4b), suggesting that a weaker ionic composition does not favor aggregation either.

**Conclusion** – We have presented a minimal aggregation model of coalescing spherical flocs under the influence of surface potential. Attempts are made to explain the physical implications of the ‘collision factor’ a term widely used but scarcely understood in engineering literature [4]. Predictions about the floc aggregate size, at various fluid and surface potential parameters, have been made using numerical simulations. These predictions are being currently validated with a series of experiments, e.g., those detailing changes in the floc sizes at various ionic composition of the static fluid, or with different polymer coated floc surfaces.

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